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INTERNATIONAL STUDY ON *ARTEMIA.** XVIII. THE HATCHING RATE OF *ARTEMIA* CYSTS – A COMPARATIVE STUDY

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ABSTRACT

A comparative study has been carried out on the hatching rate of Artemia *cysts of different geographical origin and on different batches of the same source. Substantial variation, among as well as within geographical strains, indicates that the hatching-rate criterion is not strain specific.*

In an attempt to identify the parameters which influence the hatching rate, it was found that the cyst-drying technique as well as the storage conditions may significantly affect this criterion. It has also been demonstrated that the cysts' hatchability is influenced by their previous drying and storing conditions. Optimal hatching results are guaranteed when a fluidized bed dryer is used and the cysts are stored under vacuum or nitrogen.

INTRODUCTION

Presently brine shrimp cysts are commercially available from natural sources in Argentina, Australia, Brazil, Canada, the People's Republic of China and the USA (Sorgeloos, 1980*a*).

From the limited literature on this subject it is known that the hatching rates of *Artemia* cysts vary from one strain to another (Sorgeloos and Persoone, 1975; Person-Le Ruyet and Salaun, 1977; Smith *et al.*, 1978).

According to the results obtained by Smith *et al.* (1978), even variation among batches from the same strain may be expected. This complicates the exact timing for

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the maximized harvest of instar I nauplii, which is critical both in fundamental research, e.g. in ecotoxicological studies (Sorgeloos *et al.*, 1979; Vanhaecke *et al.*, 1980), and in aquaculture (Benijts *et al.*, 1976; Sorgeloos, 1980*b*).

Presently it is not clear whether the hatching rate is strain specific, as are several other cyst characteristics (Vanhaecke and Sorgeloos, 1980), or to what extent cyst processing and/or storage conditions are involved. A better knowledge of the parameters that influence the hatching rate might lead to improved techniques in the development of better *Artemia*-cyst products.

In this regard hatching-rate analyses on cyst samples from 16 geographical strains and on samples from the same strain but harvested at different moments of the year or exposed to different processing and drying treatments have been carried out. The effect of the latter treatments on hatching efficiency was also tested.

MATERIALS AND METHODS

Cysts from 16 geographical strains of *Artemia* were examined. Details of their origin are given in Table 1. For the strains from Macau and San Francisco Bay different batches were studied.

TABLE 1
Artemia strains and batches examined

Source of cysts	*Batch number or year of harvest*
Argentina, Buenos Aires	1977
Australia, Port Alma	–
Australia, Shark Bay	no. 114
Brazil, Macau	May 1978
Brazil, Macau	November 1978 (no. 871172)
Brazil, Macau	December 1978 (no. 872112)
Brazil, Macau	no. 87500
Canada, Chaplin Lake	1979
Colombia, Galera Zamba	1977
France, Lavalduc	1979
India, Tuticorin	–
Italy, Margherita di Savoia	1977
People's Republic of China, Tientsin	1979
Philippines, Barotac Nuevo	1978
Puerto Rico, Bahia Salinas	–
USA, Great Salt Lake	1977
USA, San Francisco Bay	no. 288-2596
USA, San Francisco Bay	no. 288-2606
USA, San Francisco Bay	no. 236-2016
USA, San Pablo Bay	no. 1628
Venezuela, Port Araya	1978

For each cyst sample two cylindro-conical tubes were set up, with each 250-mg cyst in 100 ml natural seawater (35‰). The hatching tubes were incubated at 25°C and exposed to a light intensity of 1000 lux. The cysts were kept in suspension by gentle air-bubbling. At intervals of 1 h, four 250-µl subsamples were taken from the duplicate tubes with an automatic micropipet and the number of nauplii counted. Sampling was continued until the maximum hatching efficiency was attained (predetermined using the method of Sorgeloos *et al.*, 1978), or until no increase in the number of nauplii was recorded in three consecutive series of samples.

The mean values of the four subsamples, taken every hour, were expressed as a percentage of the maximum hatching value. For each run, the mean percentages from the duplicate tubes were used to calculate the non-linear regression lines using the method of orthogonal polynomials. An analysis of variance test on these regression lines revealed the one best fitting the hatching curve.

For each curve the following time periods,from incubation on, were derived graphically: t_0 = time until appearance of the first nauplii; $t_{10,50,90}$ = time until, respectively, 10, 50 or 90% hatching is attained. The time interval $t_s = t_{90} - t_{10}$ was calculated as a measure of hatching synchrony.

Hatching efficiency data were determined according to the procedure outlined by Sorgeloos *et al.* (1978), but at 25°C instead of 30°C.

RESULTS AND DISCUSSION

Comparative analysis of different Artemia *strains*

From the hatching-time characteristics (Table 2) it is clear that the hatching rates differ considerably from one strain to another. The appearance of the first nauplii fluctuates from a minimum of 13·8 h after incubation for Bahia Salinas cysts, up to a maximum of 34 h for cysts from Tuticorin, i.e. a variation of more than 20 h.

Not only hatching rates but also hatching synchronies vary considerably from one strain to another (Table 2): e.g. Buenos Aires and Tientsin cysts both start to hatch after about 16 h of incubation, their t_{90} values, however, differ with more than 5 h. Even higher variation is recorded between Great Salt Lake and Chaplin Lake cysts, i.e. for a similar t_0 figure, a difference of more than 11 h is recorded for their t_{50} values.

It is interesting to note that the hatching rates of San Francisco Bay cysts differ from the values obtained for cysts originating from San Francisco Bay transplantations in Macau and Barotac Nuevo. This is a first indication that the hatching rate is not under complete genetic control but depends to some extent on the environmental conditions under which the cysts have been produced. This is also confirmed by the observation that strains belonging to the same sibling species (Bowen *et al.*, 1978) do not have similar hatching rates.

TABLE 2
Hatching-time characteristics for *Artemia* cysts from different geographical origin

Source of cysts	t_0	t_{50}	t_{90}	t_S
San Francisco Bay (batch no. 288-2596)	15·0	17·6	20·5	5·0
San Pablo Bay	13·9	17·3	20·1	5·0
Macau (batch no. 871172)	16·4	19·8	21·9	4·4
Barotac Nuevo	14·7	18·8	22·0	6·3
Great Salt Lake	14·1	17·5	21·7	7·0
Buenos Aires	16·1	19·7	22·6	5·3
Shark Bay	20·3	23·8	28·1	7·0
Margherita di Savoia	18·7	22·3	25·3	5·3
Bahia Salinas	13·8	16·6	20·1	5·8
Galera Zamba	27·8	32·5	37·5	8·9
Port Araya	21·4	34·4	39·8	15·0
Port Alma	15·8	18·0	21·9	5·7
Chaplin Lake	14·3	22·4	33·0	17·3
Tientsin	16·0	21·0	27·2	10·1
Lavalduc	19·5	24·4	30·5	10·0
Tuticorin	34·0	43·6	48·3	10·4

t_0 = time until appearance of the first nauplii.
t_{50} = time until 50% hatching is attained.
t_{90} = time until 90% hatching is attained.
$t_S = t_{90} - t_{10}$.

Since there is found to be no correlation either between hatching rates and cyst biometrics (Vanhaecke and Sorgeloos, 1980) or between hatching rates and the amounts of energy consumed during the hatching process (Vanhaecke *et al.*, 1982), it would appear that the hatching rate of *Artemia* cysts does not directly depend on the energy content of the cysts.

When considering the practical use of cysts in aquaculture hatcheries, those needing only a short incubation time and assuring a high hatching synchrony are most interesting.

Comparative analysis of different harvests from two strains

Three harvests of San Francisco Bay and four harvests of Macau cysts reveal important differences in hatching rate among harvests within the same strain (Fig. 1). The variation in the Macau batches is lowest at the onset of hatching; but a 7-h variation in the t_{90} values is observed. In the San Francisco Bay samples on the other hand, hatching synchronies are fairly constant, but up to 10-h variation in time of commencement of hatching exists among batches.

It is evident from these data that the hatching-rate criterion is not strain specific. Variation within strains may be the result of temporal variation due to changing abiotic and biotic conditions in the salt ponds. Bohra (1980) and Sarasquete Reiriz (1979) reported variations in the hatching rate depending upon the season in which the cysts

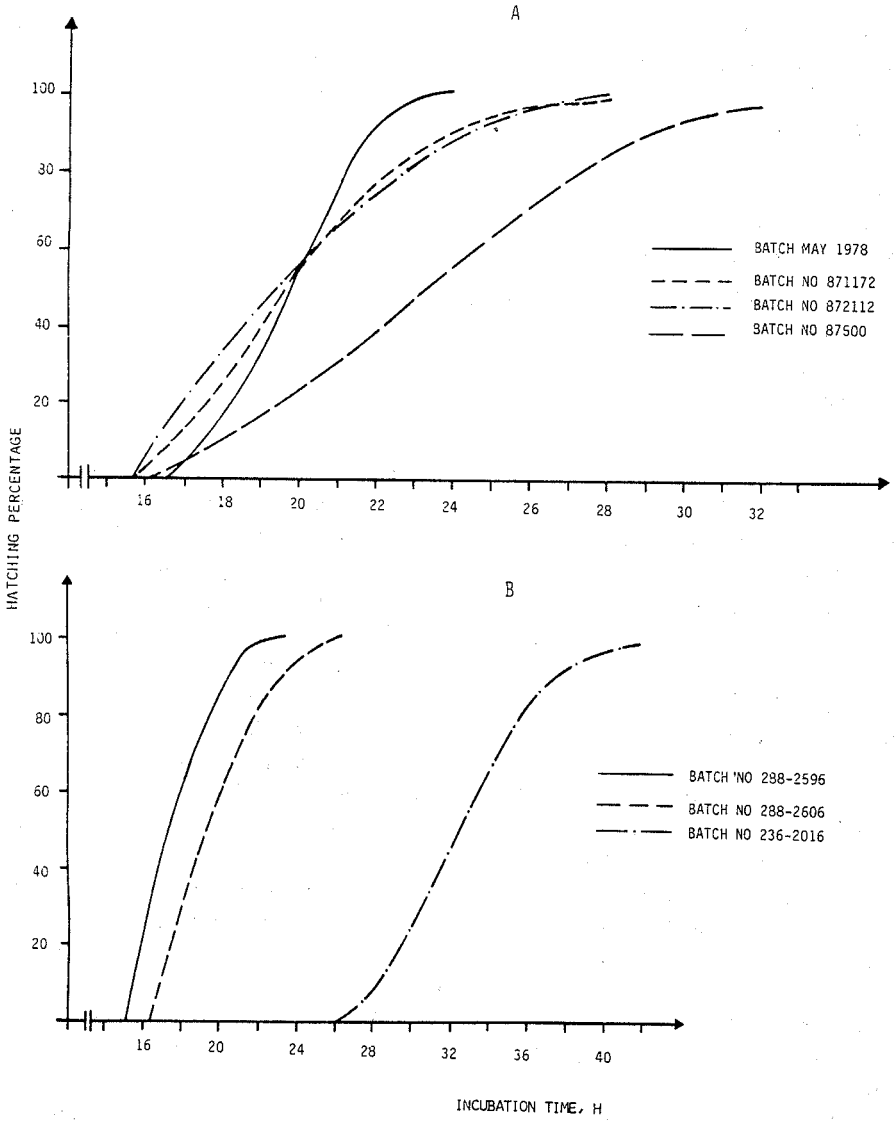

Fig. 1. Hatching curves for different batches of cysts from Macau (A) and San Francisco Bay (B).

had been produced. Variation among cyst batches can, however, also be due to different harvesting techniques. Indeed, comparing cysts collected in brine and those harvested from the shore, the latter may have been subjected to repeated hydration-dehydration cycles which certainly affect the hatching rate (Morris, 1971; Sorgeloos *et al.*, 1976).

The variability in the hatching rate among cyst batches implies that the hatching rate of a new batch of cysts should be checked to determine the most appropriate time schedule for instar I production, in order to ensure the optimal use of *Artemia* cysts in aquaculture hatcheries.

Effect of drying techniques

Five subsamples of a homogeneous batch of unprocessed Lavalduc cysts were dried under the following conditions:

- fluidized-bed dryer at 35°C (2 h drying time);
- oven dryer at 30°C; thickness of cyst layer: 0·5 cm and 1·5 cm (48 h drying time);
- oven dryer at 38°C; thickness of cyst layer: 0·5 cm and 1·5 cm (48 h drying time).

The hatching curves are graphically represented in Fig. 2 and the hatching efficiency data are given in Table 3. All the dried cysts, except for the series oven-dried in the 1·5 cm thick layer, were analyzed again after vacuum storage for one month.

The findings indicate that the hatching performances are very similar for fluidized-bed dried and unprocessed cysts. The variation in hatching efficiency among the oven-dried series is related to differences in the rate of water removal. It is obvious that the more slowly water is removed (still 40% water content after 48 h drying in a 1·5 cm thick layer at 30°C against only 4% for the 0·5 cm, 38°C series) the more the cyst's viability decreases. Apparently some of the low-drying cysts had been exposed for too long a period to water levels, exceeding 65%, that assure active cyst metabolism (Clegg and Cavagnaro, 1976). Subsequent dehydration of these cysts below the critical water level does not result in an otherwise reversible interruption of the hatching metabolism (Morris, 1971) but in the killing of the embryos. From the hatching rate data it also appears that in all oven-dried series, dehydration is not fast enough. Variation of the hatching rate as a function of the storage time under vacuum, provides the proof that during the drying process these cysts had been exposed to a hydration-dehydration cycle. As compared to the control, the t_{50} values are indeed shortened when the cysts are incubated immediately after such a cycle (Morris, 1971), but are significantly prolonged after vacuum storage of the cysts for one month (Sorgeloos *et al.*, 1976; Benijts *et al.*, 1977).

In the fluidized-bed dried cysts, on the other hand, the water content dropped below 10% after less than 2 h of treatment. As a result, neither the hatching rate (prior to and after storage) nor the hatching efficiency different significantly from the respective values obtained for untreated cysts.

Effect of storage conditions

San Francisco Bay cysts (batch no. 288-2596) and Macau cysts (batch no. 971051) were stored under vacuum, air, nitrogen, oxygen and in saturated NaCl brine for 1 and 2 years, respectively.

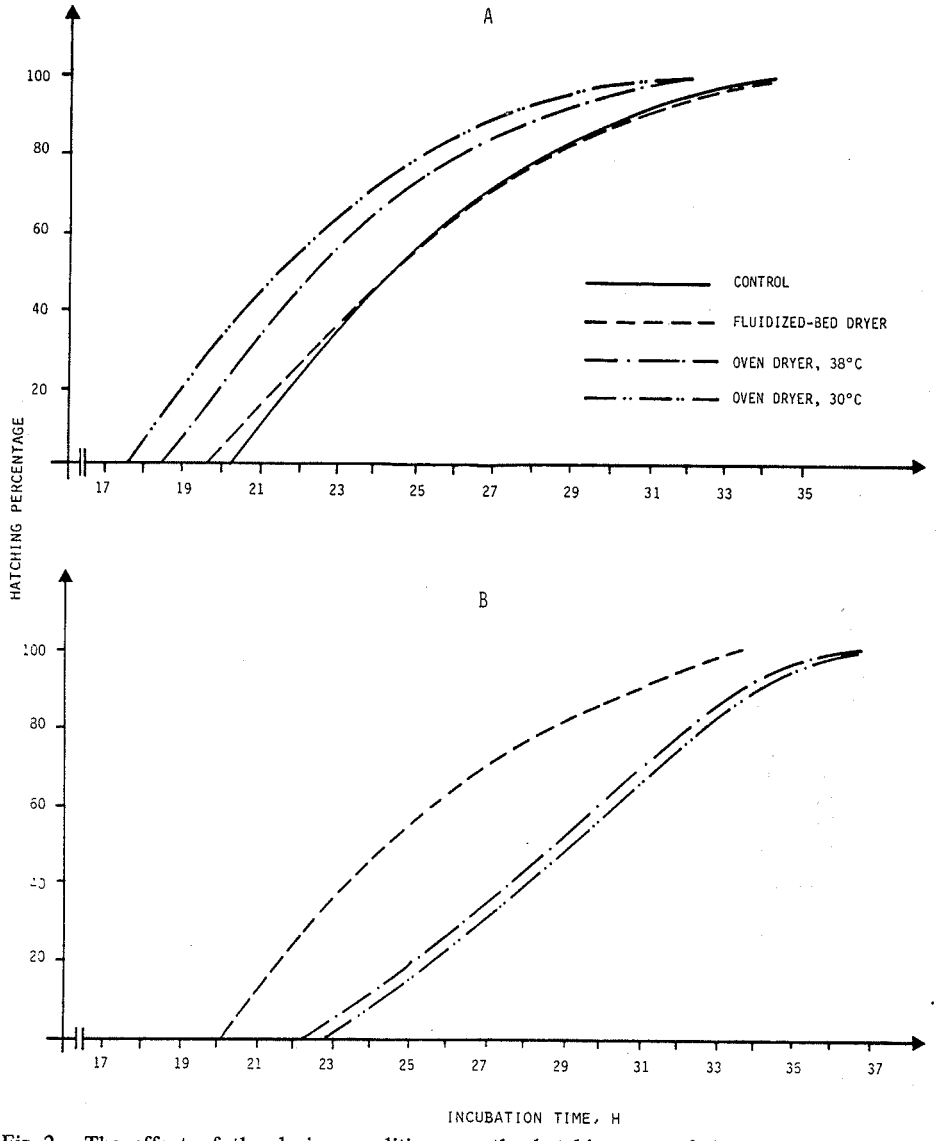

Fig. 2. The effect of the drying conditions on the hatching rate of *Artemia* cysts. A, cysts incubated immediately after drying; B, cysts incubated after vacuum storage for one month.

The hatchability data in Table 4 reveal that storage under vacuum or nitrogen does not significantly affect the hatching efficiency. The presence of oxygen in the storage medium results, however, in a substantial decrease in hatchability. According to Crowe (1971) and Crowe and Clegg (1973) this is caused by the formation of highly detri-

TABLE 3
The effect of drying conditions on the hatching efficiency of Lavalduc cysts

Drying conditions			*Hatching efficiency (nauplii/g cysts)*	
Method	*Temperature (°C)*	*Thickness of cyst layer (cm)*	$\bar{x}$	$s_{\bar{x}}$
Oven drier	30	1·5	69 120	9 760
	30	0·5	149 600 (154 120)[a]	10 240 (7 600)
	38	1·5	150 880	7 200
	38	0·5	181 360 (179 200)	9 600 (10 100)
Fluidized-bed drier	35		182 400 (181 960)	6 400 (6 920)
Control (unprocessed cysts)			178 640	(8 840)

[a] In parentheses data for same cysts but after one month storage under vacuum.

TABLE 4
The effect of different storage conditions on the hatchability[a] of *Artemia* cysts from two localities

Storage conditions	*San Francisco Bay (1 year storage)*	*Macau cysts (2 years storage)*
Oxygen	70	56
Air	–	83
Nitrogen	101	91
Vacuum	100	98
Brine 20°C	66	74
Brine −20°C	76	–

[a] Expressed as a percentage of the result obtained with the original sample.

mental free radicals. Storage in brine also leads to a significant drop in hatchability probably due to the relatively high water content of cysts stored in NaCl brine (approximately 20%; Clegg, 1978). Indeed, Clegg and Cavagnaro (1976) noted indications of enzyme activity and a significant drop in the ATP concentration at 10–35% water content. At freezing temperatures, the metabolic processes in the cysts stored in brine are greatly retarded, which might explain their lower decrease in viability as compared to the cysts stored at 20°C.

From the hatching curves of the San Francisco Bay cysts it appears that the storage conditions also influence cyst hatching rate (Fig. 3). The presence of oxygen again appears to have the most retarding effect.

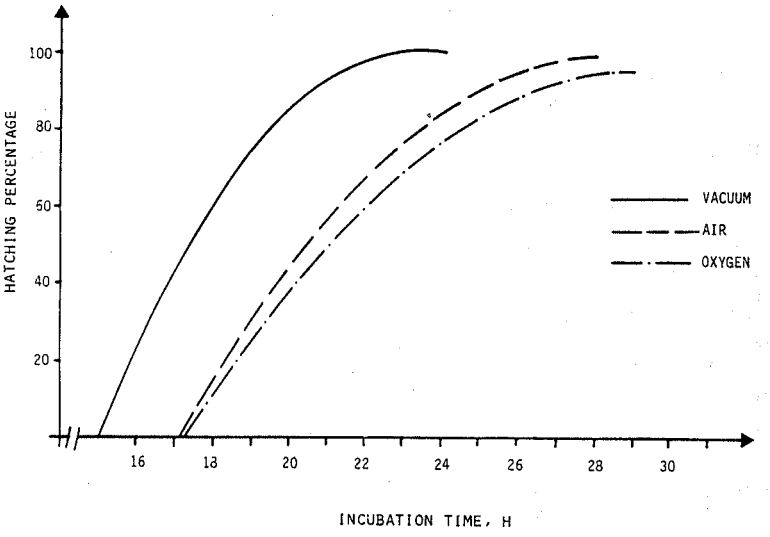

Fig. 3. The effect of storage conditions on the hatching rate of *Artemia* cysts.

CONCLUSIONS

1. The hatching rate is an important criterion in the evaluation of the overall hatching quality of *Artemia* cyst samples.
2. The hatching rate is not only a function of the geographical origin of the cyst material but is to a very large extent masked by the harvesting, processing and storage conditions.
3. In order to ensure the optimal use of *Artemia* in aquaculture hatcheries, the hatching rate of a new batch of cysts should be determined prior to use.
4. The optimal hatching quality of *Artemia* cysts can be ensured by harvesting the cysts from the water, by processing the cysts in a fluidized-bed dryer and by storaging the cysts under either vacuum or nitrogen.

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REFERENCES

Benijts, F., Vanvoorden, E. & Sorgeloos, P. (1976). Changes in the biochemical composition of the early larval stages of the brine shrimp, *Artemia salina* L. In: *Proc. 10th European Symposium on Marine Biology – 1*, eds G. Persoone & E. Jaspers, Universa Press, Wetteren, pp. 1–9.

Benijts, F., Vandeputte, G. & Sorgeloos, P. (1977). Energetic aspects of the metabolism of hydrated *Artemia* cysts. In: *Fundamental and Applied Research on the Brine Shrimp,* Artemia salina *(L.), in Belgium*, eds E. Jaspers & G. Persoone, EMS Special Publication No. 2, Institute for Marine Scientific Research, Bredene, Belgium, pp. 79–87.

Bohra, O. P. (1980). A note on *Artemia* culture from a local strain: Abstract. In: *The Brine Shrimp* Artemia – *3*, eds G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers. Universa Press, Wetteren, p. 131.

Bowen, S. T., Durkin, J. P., Sterling, G. & Clark, L. S. (1978). *Artemia* hemoglobins: genetic variation in parthenogenetic and zygogenetic populations. *Biol. Bull.*, **155**, 273–87.

Clegg, J. S. (1978). Interrelations between water and cellular metabolism in *Artemia* cysts. VIII. Sorption isotherms and derived thermodynamic quantities. *J. biophys. biochem. Cytol.*, **94** (2), pp. 123–38.

Clegg, J. S. & Cavagnaro, J. (1976). Interrelationships between water and cellular metabolism in *Artemia* cysts. IV. ATP and cyst hydration. *J. biophys. biochem. Cytol.*, **88** (2), 159–66.

Crowe, J. H. (1971). Anhydrobiosis: an unsolved problem. *Am. Nat.*, **105**, 563–74.

Crowe, J. H. & Clegg, J. S. (1973). *Anhydrobiosis*, Dowden, Hutchinson & Ross, Stroudsburg, USA.

Morris, J. E. (1971). Hydration, its reversibility, and the beginning of development in the brine shrimp, *Artemia salina. Comp. Biochem. Physiol.*, **39A**, 843–57.

Person-Le Ruyet, J. & Salaun, A. (1977). Etude comparative des possibilités d'élevage larvaire de quelques poissons marins avec une souche d'oeufs d'*Artemia salina* de Chypre. *ICES-CM,* **32**, 13 pp.

Sarasquete Reiriz, M. del C. (1979). Estudio de los quistes de *Artemia salina* de las salinas de Cadiz. *Thesis*, University of Santiago de Compostela, Spain.

Smith, T. I. J., Hopkins, J. S. & Sandifer, P. A. (1978). Development of a large-scale *Artemia* hatching system utilizing recirculated water. In: *Proc. 9th Ann. Meeting WMS*, ed. J. W. Avult, Jr., Louisiana State University, Baton Rouge, pp. 701–14.

Sorgeloos, P. (1980*a*). Improvements in availability and use of *Artemia* as food source for *Macrobrachium*. In: *Grant Prawn, 1980, Bangkok*, IFS Provisional Report No. 9, International Foundation for Science, Stockholm, pp. 123–32.

Sorgeloos, P. (1980*b*). The use of the brine shrimp *Artemia* in aquaculture. In: *The Brine Shrimp* Artemia – *3*, eds G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers, Universa Press, Wetteren, pp. 25–46.

Sorgeloos, P. & Persoone, G. (1975). Technological improvements for the cultivation of invertebrates as food for fishes and crustaceans. II. Hatching and culturing of the brine shrimp, *Artemia salina* L. *Aquaculture*, **6**, 303–17.

Sorgeloos, P., Baeza-Mesa, M., Benijts, F. & Persoone, G. (1976). Current research on the culturing of the brine shrimp *Artemia salina* L. at the State University of Ghent, Belgium. In: *Proc. 10th European Symposium on Marine Biology – 1*, eds G. Persoone & E. Jaspers, Universa Press, Wetteren, pp. 473–95.

Sorgeloos, P., Persoone, G., Baeza-Mesa, M., Bossuyt, E. & Bruggeman, E. (1978). The use of *Artemia* cysts in aquaculture: the concept of 'hatching efficiency' and description of a new method for cyst processing. In: *Proc. 9th Ann. Meeting WMS*, ed. J. W. Avault, Jr., Louisiana State University, Baton Rouge, pp. 715–21.

Sorgeloos, P., Remice-Van der Wielen, C. & Persoone, G. (1979). The use of *Artemia* nauplii for toxicity tests – a critical analysis. *Ecotoxicology and Environmental Safety*, **2**, 249–55.

Vanhaecke, P., Persoone, G., Claus, C. & Sorgeloos, P. (1980). Research on the development of a short term standard toxicity test with *Artemia* nauplii. In: *The Brine Shrimp* Artemia – *1*, eds G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers, Universa Press, Wetteren, pp. 263–85.

Vanhaecke, P. & Sorgeloos, P. (1980). International study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. In: *The Brine Shrimp* Artemia – *3*, eds G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers, Universa Press, Wetteren, pp. 393–405.

Vanhaecke, P., Lavens, P. & Sorgeloos. P. (1982). International study on *Artemia*. XX. Energy consumption in cysts and early larval stages of various geographical strains of *Artemia* (submitted for publication).